CLAIMS

1. An *in vitro* diagnostic method for quantification of a clinical chemistry analyte from a clinical sample wherein the clinical chemistry analyte

a) undergoes a chemical reaction or reactions with a reagent or reagents in one or several steps, or in a reaction sequence, or

b) catalyses a chemical reaction, or reactions, or a reaction in a reaction sequence of a reagent or reagents, in one or several steps;

in a reaction system, said reaction or reactions or reaction sequence resulting in a change of a measurable property of a compound or compounds of said reaction or reactions or reaction sequence **characterized** in that

i) said chemical reaction or reactions or reaction sequence results in

- formation of a two-photon fluorescent compound, or
- a change in two-photon fluorescence properties of the reaction system comprising at least one two-photon fluorescent compound; and

ii) said analyte is quantified by exciting said two-photon fluorescent compound or compounds and measuring two-photon exited fluorescence, and relating said measured fluorescence to method standardization data based on measurements obtained from reference material of said analyte.

2. The method of claim 1, **characterized** in that it comprises the steps of

a) bringing the clinical sample comprising the clinical chemistry analyte in contact with a specific assay reagent or reagents;

b) allowing, in the reaction system, said analyte to undergo a chemical reaction with said reagent or reagents, or allowing said analyte to catalyze a chemical reaction or reactions of said reagent or reagents;

c) optionally repeating steps a) and b) one or several times;

d) said reaction or reactions of step or steps b) resulting in formation of a two-photon fluorescent compound, or resulting in a change in two-photon

fluorescence properties of said reaction system comprising at least one two-photon fluorescent compound; and

e) quantifying said analyte by exciting said two-photon fluorescent compound or compounds, measuring two-photon excited fluorescence, and relating said measured fluorescence to method standardization data based on measurements obtained from reference material of said analyte.

3. The method according to claim 1 or 2, **characterized** in that the fluorescence resulting from two-photon fluorescence excitation is measured kinetically.

4. The method according to claim 1 or 2, **characterized** in that the fluorescence resulting from two-photon fluorescence excitation is measured as an end-point signal.

5. The method according to any of claims 2 to 4, **characterized** in that the quantification of the clinical chemistry analyte is carried out for several samples by repeating the steps a) to e) of claim 2 for each sample.

6. The method according to any of claims 2 to 5, **characterized** in that several clinical chemistry analytes are quantified by repeating the steps a) to e) of claim 2 for each analyte.

7. The method according to any of claims 1 to 5 **characterised** in that the clinical chemistry analyte or analytes are selected from the group consisting of albumin, total protein, hemoglobin, ammonia, carbonate, bilirubin direct, bilirubin total, calcium, chloride, iron, magnesium, phosphate, cholesterol HDL, cholesterol LDL, cholesterol total, creatinine, fructosamine, glucose, lactate, triglycerides, urea, uric acid, acid phosphatase, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, amylase pancreatic, amylase total, cholin esterase, creatine kinase, glutamyl transferase, glutamate dehydrogenase, hydroxybutyrate dehydrogenase, lactate dehydrogenase and lipase.

8. Use of a fluorometric device employing two-photon fluorescence excitation for *in vitro* diagnostic quantification of a clinical chemistry analyte or analytes from a clinical sample or samples, wherein said quantification of one or more of said analytes comprises one or more chemical reactions resulting in formation of at least one two-photon fluorescent compound, or a change in two-photon fluorescence properties of the reaction system comprising at least one two-photon fluorescent compound.

9. The use according to claim 8 **characterized** in that the device comprises a pulse laser for two-photon fluorescence excitation with

a) a pulse length shorter than 10 nanoseconds,

b) with pulse repetition frequency higher than 10 kHz,

c) TEM 00 mode polarized beam output, and

d) average beam power in the sample from 20 to 200 mW, preferably 85 to 120 mW and most preferably about 100 mW.

10. The use according to claims 8 or 9 **characterized** in that the clinical chemistry analyte or analytes are selected from the group consisting of albumin, total protein, hemoglobin, ammonia, carbonate, bilirubin direct, bilirubin total, calcium, chloride, iron, magnesium, phosphate, cholesterol HDL, cholesterol LDL, cholesterol total, creatinine, fructosamine, glucose, lactate, triglycerides, urea, uric acid, acid phosphatase, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, amylase pancreatic, amylase total, cholin esterase, creatine kinase, glutamyl transferase, glutamate dehydrogenase, hydroxybutyrate dehydrogenase, lactate dehydrogenase and lipase.

11. The use according to claim 10, **characterized** in that the same fluorometric device is consecutively used for the quantification of at least 2, preferably 5, more preferably 10, even more preferably 20 and most preferably all of the clinical chemistry analytes of the group.

12. The use according to any of claims 8 to 11, **characterized** in that the same fluorometric device is also used for the quantification of bioaffinity analytes.

13. A system for *in vitro* diagnostic quantification of at least one clinical chemistry analyte from a clinical sample or samples, **characterized** in that the system comprises

a) a fluorometric device employing two-photon excited fluorescence for quantifying one or several clinical chemistry analytes, and

b) a data processing unit with software for dedicated data reduction for said quantification of said analyte or analytes using said fluorometric device, wherein said quantification of one or more of said analytes comprises one or more chemical reactions resulting in formation of at least one two-photon fluorescent compound, or a change in two-photon fluorescence properties of the reaction system comprising at least one two-photon fluorescent compound.

14. The system of claim 13, **characterized** in that it comprises test cuvette magazines holding the sample tubes and test cuvettes.

15. The system of claim 13 or 14, **characterized** in that it comprises a dilutor dispenser for diluting the sample and for dispensing it into the test cuvette.

16. The system of claim 13, 14 or 15, **characterized** in that it comprises mechanics for moving the test cuvettes and/or the dispensing head.

17. The system of according to any of claims 13 to 16, **characterized** in that it comprises a control unit for automatic control of the system.

18. A software product for a diagnostic quantification system to any of claims 13 to 17, **characterized** in that the software product comprises means for controlling a processing unit of the quantification system to execute or control step e) of claim 2.

19. The software product according to claim 18, **characterized** in that it additionally comprises means for controlling the processing unit of the quantification system to execute or control any combination of one or more steps a) to d) of claim 2.